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COMPARATIVE MORPHOLOGY OF HAEMOCYTE FROM TWO SPECIES OF GIANT AFRICAN LAND SNAILS (*Archachatina marginata* AND *Achatina achatina)*

J.A. ABIONA[1]*, P.A. AKINDUTI[2], P. A., OYEKUNLE[2], O. A. OSINOWO[1] AND O.M. ONAGBESAN[1].

[1]Department of Animal Physiology, Federal University of Agriculture, Abeokuta.
[2]Department of Veterinary microbiology, Federal University of Agriculture, Abeokuta

***Corresponding author:** abionajohn@yahoo.ca;

ABSTRACT

A study was conducted to evaluate the presence and types of haemocytes inherent in haemolymph of two species of giant African land snails (*Archachatina marginata* and *Achatina achatina*). Haemolymph samples were obtained from three liveweight groups of snails (< 100 g, 101-150 g and >150 g) after removal of the first three whorls of the shell. Smears were made from thin layer of settled portion of the haemolymph after six hours. Staining was carried out after air drying at room temperature with May-Grünwald-Giemsa stain. Four replicates per liveweight per species were used. Representative slides were selected after viewing under microscope. Dimensions of haemocytes identified were taken followed by photomicrograph. Results showed that four haemocyte types were present in the haemolymph of both species. Those haemocytes identified in *Archachatina marginata* had significantly (P<0.001) higher dimensions than those found in *Achatina achatina.* Morphologically, the haemocytes were different in terms of shapes, nucleus position and cytoplasm types. It can therefore be concluded from this study that four circulating haemocytes types are present in both *Archachatina marginata* and *Achatina achatina*. Furthermore, in terms of dimension, *Archachatina marginata* had higher values compared to *Achatina achatina*. Also, morphologically, those four haemocyte types are different from each other in both specie of giant African land snails.

Key words: Giant African land snails, haemocytes and cytoplasm types.

INTRODUCTION

Haemocytes constitute a major internal defense mechanism of the gastropod mollusk. They are responsible for recognition and destruction of many varieties of pathogens (Nadya *et al*., 2006). Haemocytes are the invertebrate analogue of blood cells found in mammals. They are known to perform a lot of roles in invertebrates especially in snails. Notable among the roles are agglutinins production which are responsible for prompt elimination of bacterial in the system of the animal (Richards and Renwrantz, 1991). In mollusc generally, lectins synthesized by haemocytes are either released into the haemolymph or expressed on the surface

of the circulating haemocytes, where they are able to act as cyto-phylic receptors (Richards and Renwrantz, 1991). Mechanisms such as opsonization, internalization or endocytosis are also powerful mechanism for destruction of microorganism and elimination of microbial assault (Prieur *et al*., 1990 and Canesi *et al*., 2002a). Other mechanism used are; haemolymph coagulation (Iwanaga *et al*., 1978), expression of antimicrobial peptides, lectin complement pathway mediated by bacterial cell wall components (Fujita, 2002; Iwasaki and Medzhitov, 2004). Toll-like receptor-mediated antimicrobial peptide production is also reported in invertebrate defense mechanism (Lemaitre *et al*., 1996; Imuler and Hoffmann, 2000; Krutzik *et al*., 2001). It therefore becomes very important that those body guides should be identified and classified especially in giant African land snails in which such information is not available. Constant check of the number and types of haemocytes present in the body of this animal can go a long way in determining their immune status, especially for those snails that are reared indoor. This study therefore aimed at evaluating the presence and identifies the different haemocytes types present in the haemolymph of giant African land snails (*Archachatina marginata* and *Achatina achatina).*

MATERIALS AND METHODS

Experimental site

The study was conducted at the Veterinary Microbiology Laboratory, University of Agriculture, Abeokuta, Nigeria. The location lies within the rainforest belt of Western Nigeria, latitude 7°14' 02.86"N, longitude 3°26' 19.45"E (Google Earth, 2010) and altitude 141 masl. The climate is humid with a mean annual rainfall of 1037 mm. The annual mean temperature and humidity are 34.7°C and 82% respectively.

Experimental animals and their management

A total of 24 snails (12, *Archachatina marginata* and 12, *Achatina achatina*) were used for all the studies. Both species were further divided into three liveweight groups (<100 g, 101-150 g and > 150 g). The animals were kept in plastic cages with dimension 34 cm x 22 cm x 25 cm. They were fed with a mixture of chicken layer's mash and dried paw-paw leaves (1:1, w/w) *ad libitum*. Water was provided *ad libitum.*

Dissection and haemolymph collection

Four replicate per liveweight per species were selected and the first three whorls of the snails were removed to expose the mantle cavity for easy drainage of the haemolymph. Thereafter, the cavity wall was broken and the haemolymph drained into sterilized 100 ml beaker for each snail and allowed to settle for six hours.

Haemocyte collection

After six hours, the haemolymph were gently decanted until white thin layer were reached. The thin layer was further allowed to settle for thirty minutes before decanted further to ensure high haemocyte concentration at the base of the glass ware.

Slide preparation for morphological studies

Smears were made on 22 x 22 mm slide from the concentrated thin layer of haemocytes at the base of the glass wares. The slides were air dried at room temperature and stained with MayGrünwald-Giemsa (Delgado *et al*., 2001). Representative slides with clear haemocyte population were chosen and observations were made using Camera attached to a microscope and photomi-

crographs were taken at X 400 magnification.

RESULTS

The summary of least-squares analysis of variance on the effect of species on the dimension of haemocytes in giant African land snails is presented in Table 1. Results showed that four haemocytes types were found in both species (Type 1, Type 2, Type 3 and Type 4). Species effect was highly significant (P<0.001) on the four haemocyte types identified (Table 1).

Table 1: Summary of analyses of variance for haemocyte dimension of giant African land snails (*Archachatina marginata* and *Achatina achatina*)

Source	Df	Mean squares			
		Type 1	Type 2	Type 3	Type 4
Species	1	534.727***	1562.500***	382.852***	93.789***
Error	38	36.149	16.299	6.034	4.044

***P<0.001

Least-squares means showing effect of species on haemocyte diameter are presented in Table 2. For all the four types of haemocytes observed in both species, *Archachatina marginata* had higher diameters compared to those of *Achatina achatina*. For Type 1, *Archachatina marginata* had a mean diameter of 14.438 ± 1.344 μm as compared to *Achatina achatina* with 7.125 ± 1.344μm. Similarly, for Type 2, *Archachatina marginata* recorded a value of 22.875 ± 0.903 μm compared to *Achatina achatina* with mean diameter of 10.375 ± 0.903 μm. Type 3 and Type 4 also followed the same trend for both *Archachatina marginata* and *Achatina achatina* (11.125 ± 0.549 μm vs 4.938 ± 0.549 μm) and (5.125 ± 0.450 μm vs 2.063 ± 0.450 μm).

Table 2: Least – squares means for haemocyte dimension of giant Africa land snails *Archachatina marginata* and *Achatina achatina*)

	Haemocyte Diameter			
Species	**Type 1 (μm)**	**Type 2 (μm)**	**Type 3 (μm)**	**Type 4 (μm)**
Archachatina marginata	14.438 ±1.344a	22.875 ± 0.903a	11.125 ± 0.549a	5.125 ± 0.450a
Achatina achatina	7.125 ±1.344b	10.375± 0.903b	4.938± 0.549b	2.063 ± 0.450b

[a,b] Means in the same column with different superscripts differ significantly (P<0.001)

Plates 1, 2 and 3 show the different types of haemocyte present in the haemolymph of giant African land snails. The morphology of the different haemocyte types are shown in Table 3. The Type 1 haemocyte had an oval shape with nucleus at the base of the cell coupled with vacuolated cytoplasm, while Type 2, haemocyte had a shape that was lobulated, with branched nucleus both at the sides and centre of the cell. Type 3 was also observed to have round shape with a non – visible nucleus with vacuolated cytoplasm. Type 4 haemocyte was also seen to have a round shape with nucleus at the centre surrounded by a freely dispersed cytoplasm.

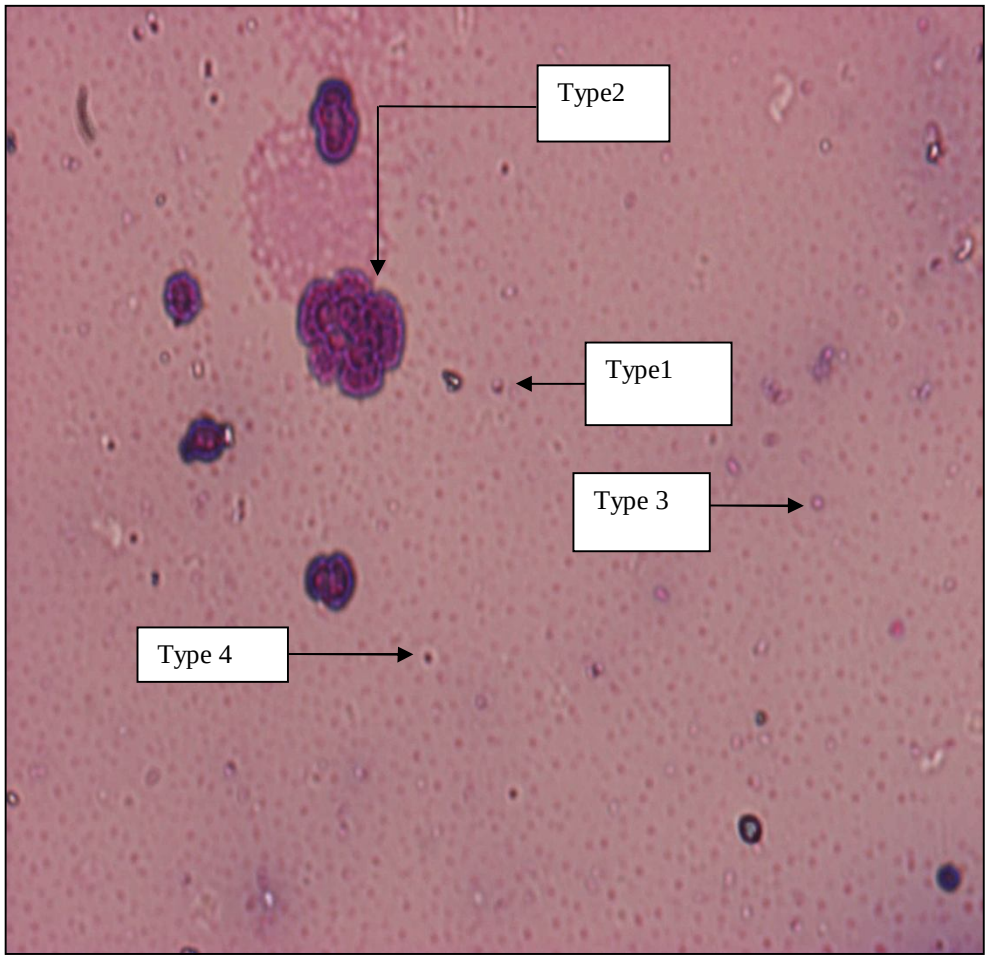

Plate 1: Photomicrograph of different haemocytes types observed in haemolymph of *A. achatina* (x 400)

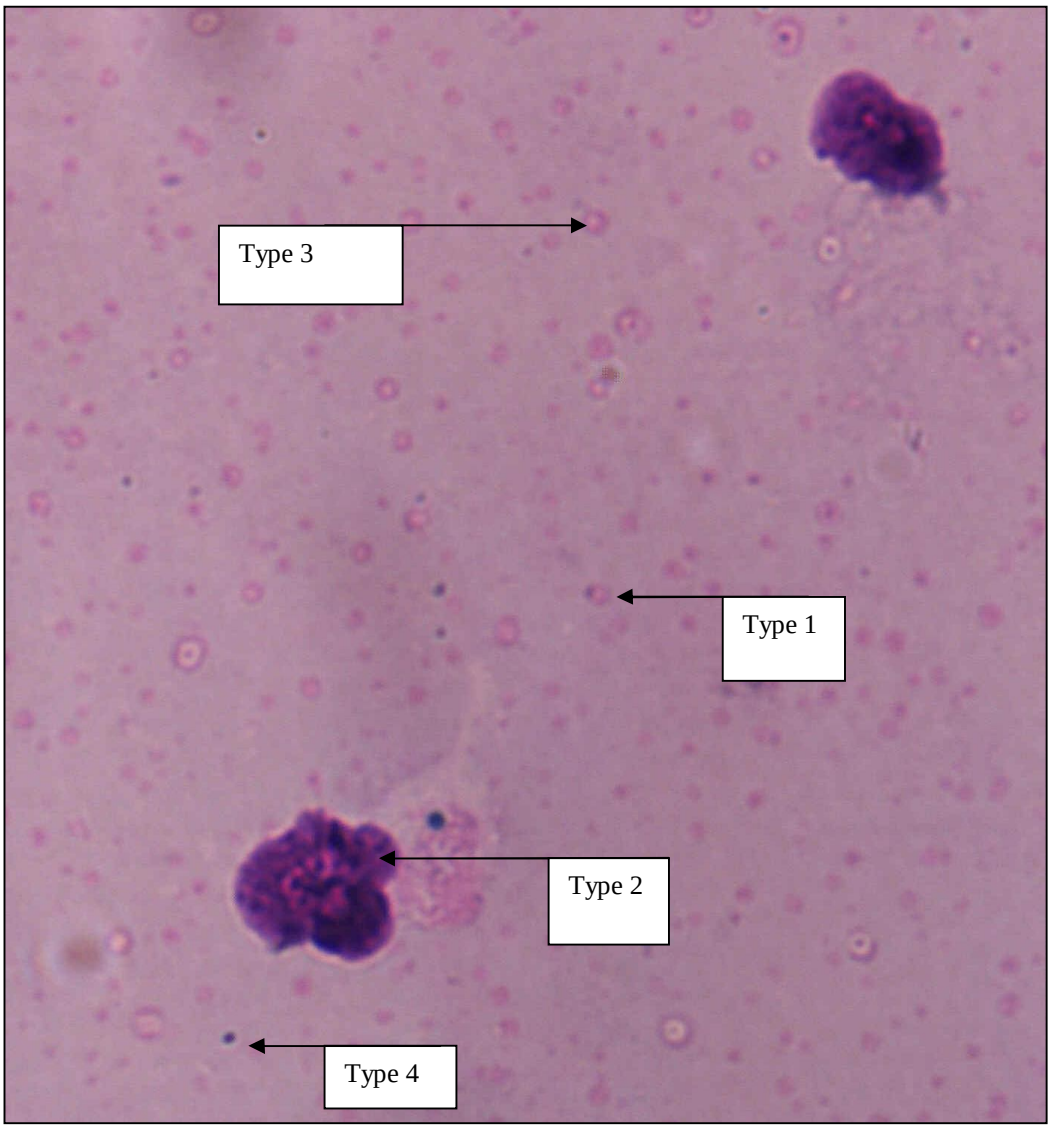

Plate 2: Photomicrograph of different haemocytes types found in haemolymph of *A. marginata* (x 400)

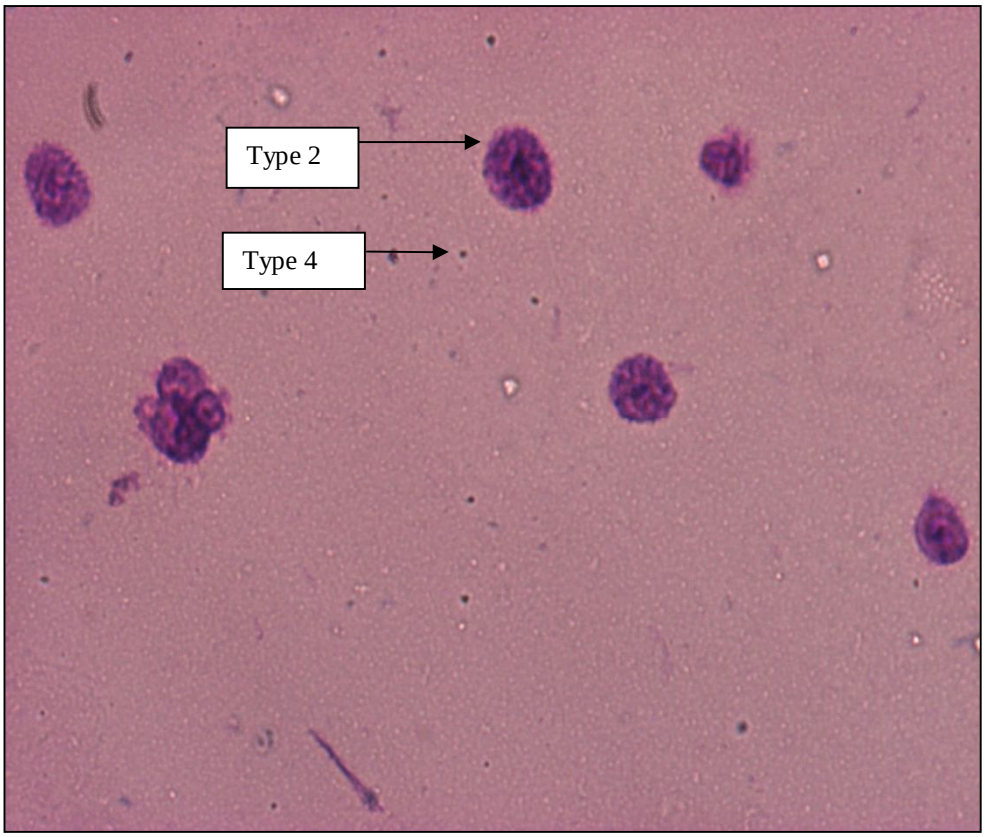

Plate 3 : Photomicrograph of different haemocytes types found in haemolymph of *A. marginata* (x 400)

Table:3 Morphological description of different haemocyte types found in the haemo lymph of giant African land snails (*Archachatina marginata* and *Achatina achatina*)

Haemocyte type	Shape	Nucleus position	Cytoplasm type
Type 1	Oval	Base	Vacuolated
Type 2	Lobulated	Branched (both side and centre	Vacuolated
Type 3	Round	Not visible as in matured eryth-rocyte	Vacuolated
Type 4	Round	Centre	Dispersed around nucleus

DISCUSSION

The four types of haemocytes identified in this study are different to those reported by Matricon-Gondran (1990) who confirmed and described two types of haemocytes in the haemolymph of temperate species of snail (*Biomphalaria glabrata*). However, the results of this study are in agreement with the report of Delgado *et al*. (2001) who identified four haemocyte types in haemolymph of similar species previously reported by Matricon-Gondran (1990). The number of circulating cell types reported confirmed that haemocytes are heterogeneous in population in different species of snails (Barraco *et al*., 1993; Bezerra *et al*., 1997; Matricon-Godran and Letorcart, 1999; Johnston and Yoshino, 2001). Apart from diameter, haemocytes have also been distinguished based on the staining affinities of their cytoplasmic granules in which acidophilic cytoplasmic granules stained red-brown or pink (Cheng, 1975; Cheng, 1981; Suresh and Mohandas, 1990; Kumazagua *et al*. 1991). In this study, considering both species across board, the diameters of those four haemocytes identified were higher in *Archachatina marginata* compared to *Achactina achatina*. This observation is pointing to the fact that differences in pattern of their survival and adaptability to their environment which favors *Archachatina marginata* than *Achatina achatina* may be corroborated by differences in haemocyte diameter for all the four cell types found in this study. This observation may also be due to genetic differences in both species. According to Fagbuaro *et al*. (2002), both species were reported to have different chromosome numbers. Differences in their growth rate may also be a factor as it relates to production of haemocytes and other antimicrobial peptides which can improve the physiological status of the animal. Haemocytes are associated with agglutinins production which is necessary in the elimination of foreign agent in the system of the animal (Richards and Renwrantz, 1991), and then the quantity produced may be related or influenced by the size of cells producing them. Presence of erythrocyte agglutinins in the haemolymph of these two species (*A. marginata* and *A. achatina*) of snail was confirmed by Abiona *et al*. (2012) who found that haemagglutination titre of those agglutinins were more in *A. marginata* than those of Achatina. This observation is corroborating the fact that differences in haemocyte diameter may be one of the reasons for their differences in survival and adaptability to their natural environment. Considering the description of Judith *et al*. (2007) of formed element in mammalian blood, Type 1 haemocyte seen in this study resembled agranulocyte and monocytes which give rise to macrophages whose function is to act as phagocyte to bacteria, dead cells and cell parts. The Type 2 haemocytes were similar to granulocytes basophils which release histamine to attract white blood cells to the site of infection. It usually has a lobed nucleus. The Type 3 haemocytes were similar to agranulocytes mature red blood cells which have no nucleus. The Type 4 haemocytes resembled typical agranulocyte lymphocytes which attack damaged or diseased cells, with a capacity to produce antibodies. Since innate immunity involves the cooperation of both cellular and humoral defense reaction, then macrophage-like phagocytic cells called haemocytes are known to be responsible for cell mediated response and play vital role in elimination of pathogens via phagocytosis, encapsulation and production of reactive oxygen intermediates (Dikkeboom *et al*., 1987; Adema *et al*., 1993) which prevent them against microbial attack. The position of nucleus and cytoplasm type may also influence agglutinins and other antimicrobial

peptide production since they are protein in nature.

CONCLUSION

This study has affirmed the presence of four haemocyte types in the haemolymph of A. marginata and A. achatina. It was also established that both species had similar haemocyte types with differences in diameter. *Archachatina marginata* was seen to have wider haemocytes compared to *Achatina achatina*. It was concluded that this difference in dimension of the haemocyte may play a role in adaptation and survival of the snails in their natural environment.

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